In response to the Examiner's objection, applicants have cancelled claims 1-19, and added new claims 20-43. These new claims address the Examiner's objection.

Claims 1-19 are rejected are rejected under 35 USC §103(a) as being unpatentable over WO 97/37012 in view of WO 96/29411, WO 94/12650, U.S. Patent No. 5,695,977, Cruz et al. and Mazure et al.

The Examiner relies on WO '012 as teaching a process for changing the expression of a nucleic acid sequence which is present endogenously in a eukaryotic cell by transfecting the cell with a vector which comprises an expression control sequence and a first amplification gene and a positive selective marker gene flanked by recombinase site-specific sequences, which are flanked by sequences for homologous recombination. The Office Action says that the recombinase site-specific sequences may be *loxP* sequences. The Office Action also indicates that a negative selection marker may be located outside the homologous recombination sequences. WO '012 is also relied upon as disclosing that the nucleic acid located between the recombinase site-specific sequences may be excised by the transient activation of a site-specific recombinase. WO '012 is further relied upon as disclosing the expression control sequence may be a hypoxia-induced nucleic acid sequence. The Examiner refers to the abstract, the summary, the claims, the figures and pages 12-22 of WO '012.

The Examiner admits that WO '012 does not teach that the cell may be a DHFR-negative cell and the homologous flanking sequences may be *dhfr* sequences, nor that the expression control sequence may be a HIF-binding nucleic acid sequence, nor that a second vector which has a construct as described above which contains a gene encoding DHFR may be transfected into the cell.

The Examiner relies on WO '650 and WO '411 as teaching the insertion by homologous recombination of an expression control nucleic acid element into the genome of a cell adjacent to a gene of interest, wherein the construct encodes a gene for DHFR. The Examiner refers to the abstract, the summary, the figures, the claims and pages 9-13, 17-19 and 21-23 of WO '650; and the abstract, the summary, the figures, the claims and pages 19-23 of WO '411.

The Examiner relies on Cruz et al. as teaching the insertion of a construct into a host genome where the homologous flanking sequences are *dhfr* sequences, and the host cell is DHFR-negative. The Examiner refers to the entire article.

U.S. '977 is relied upon as teaching the insertion of an enhancer construct into a host genome by homologous recombination where the encoded marker sequence is flanked by recombinase sites, and where positive and negative markers facilitate the insertion, selection and marker removal process. The Examiner refers to the abstract, the claims, the figures, and columns 2-8.

Mazure et al. is relied upon as teaching the use of a construct which encodes a HIF-binding nucleic acid to control genes associated with cellular responses to hypoxia. The Examiner refers to the abstract, the introduction, the figures and materials and method section of Mazure et al.

The Examiner takes the position that it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of WO '012 with WO '411, WO '650, U.S. '977, Cruz et al. and Mazure et al. to arrive at the present invention because the entire concept of the claimed invention is considered embraced by the teachings of WO '012. Limitations such as using DHFR-negative cells with a DHFR encoding vector, and *dhfr* flanking homologous sequences to target the insertion of the construct/vector into the genome of a host cell, and the introduction of an expression control sequence such as HIF-binding nucleic acid sequences adjacent to an endogenous gene in the host genome are considered to be all well known techniques for gene manipulation in a host cell as taught by the cited references.

This rejection is respectfully traversed as to new claims 20-43. Applicants respectfully submit that the combination of the cited references would not have been made without improper hindsight reasoning.

WO '012 discloses a genetic construct comprising a first expression cassette which comprises (1) a recombinase genetic unit which comprises a genetic sequence which encodes a site-specific recombinase (for example a *cre* or *flp* gene) which is placed

upstream of a terminator sequence and operably under the control of a first promoter and (2) a transgene unit which comprises one or more expressible genes, placed operably under the control of a second promoter, wherein the recombinase genetic unit and the transgene unit are linked and the first expression cassette is flanked by two recombination loci (such as *lox* or *frt* sites) which are capable of binding to the site-specific recombinase. See page 16, line 28 through page 17, line 4, and claims 1-3.

Optionally, the first expression cassette flanked by recombination loci can be inserted into a second expression cassette comprising a second transgene, wherein the second expression cassette is separated from the first expression cassette by a spacer region. See page 21, lines 15-22. Page 22, lines 14-19, disclose that the transgene of the second expression cassette may be, *inter alia*, a selectable marker gene.

Page 23, lines 6-13, disclose that in a further embodiment of the invention, the genetic construct is suitable for integration into the genome of a cell in which it is expressed. The reference discloses that in order to achieve integration of the genetic construct into the genome of a host cell certain additional genetic sequences may be required, such as one or more left and/or right T- DNA border regions flanking the genetic sequence to be integrated.

Figure 5 shows an example of a vector plasmid for use in accordance with the WO '012 invention. The plasmid contains the Sc4 promoter, a *cre* gene and an Sc1 promoter-*nptII* gene- Sc3 terminator cassette (which seems to be a positive selection marker),

wherein the *cre* gene and the cassette are flanked by *loxP* sites in direct repeat configuration, and the plasmid further contains a promoterless *gusA-nos3'* gene cassette. Flanking the Sc4 promoter and the promoterless gene cassette are left border and right border DNA sequences. See page 34, lines 21-30.

There are several distinctions between the invention described in WO '012 and the present invention. The invention described in WO '012 to discloses a vector containing therein an expression control sequence (i.e. a transgene) and a genetic sequence which encodes a site-specific recombinase, wherein two target sequences for a site-specific recombinase are flanking the transgene and recombinase genetic units. There is no provision for a heterologous expression control sequence between the targets for site-specific recombinase. In addition, while the genetic construct according to WO '012 contains left border and right border sequences to facilitate the *in vivo* insertion of the genetic construct into plant DNA (see claim 40), there is absolutely no indication that homologous recombination is taking place, as with the present invention. Furthermore, WO '012 only describes the application of its methods in plant cells in which the integration is mediated by agrobacteria.

Page 9, final paragraph, of the present specification discloses that in contrast to the recombinant production of proteins by site-unspecific integration of heterologous genes (as in WO '012) and their associated disadvantages, the process according to the invention utilizes the advantages of site-specific endogenous gene activation by homologous recombination.

Thus, the Examiner is quite incorrect in his statement of page 6 of the Office Action that WO '012 teaches a vector comprising an expression control sequence, a first amplification gene and a positive selective marker gene flanked by recombinase site-specific sequences, **which are flanked by sequences for homologous recombination**. There is simply no disclosure whatsoever in this regard in WO '012.

WO '650 discloses a general method of targeting DNA sequences into the genomic DNA of a cell by homologous recombination. Exogenous DNA which repairs, alters, deletes or replaces a sequence present in the cell or regulatory sequences not normally functionally linked to an endogenous gene, along with DNA sequences with the genomic DNA and a selectable marker, are transfected into cell to produce homologously recombinant cells containing the construct. See, for example, claim 1. There is absolutely no disclosure in WO '650 of target sequences for a site-specific recombinase flanking the exogenous DNA, and within the homologenous DNA sequences, as claimed in the present invention.

WO '411 teaches DNA constructs comprising (a) targeting sequences; (b) a regulatory sequence; (c) an exon; and (d) an unpaired splice-donor site. As disclosed on pages 20-23 of WO '411, the targeting sequence is a DNA sequence which permits homologous recombination into the genome of the selected cell containing the gene of interest. The regulator sequence can be comprised of one or more of a variety of elements, including promoters, enhancers, scaffold-attachment regions or matrix

attachment regions, negative regulatory elements, locus control regions, transcription factor binding sites or combinations of these sequences.

The exon contains DNA which encodes one or more amino acids and/or partially encodes an amino acid. The DNA construct is designed such that, upon transcription and splicing, the reading frame is in-frame with the second or subsequent exon of the endogenous gene's coding regions.

Splice-donor sites are included to direct the splicing of one exon to another exon and thereby to remove any introns contained in the gene. An unpaired splice-donor site is a splice-donor site which is present in a target construct and is not accompanied in the targeting construct by a splice-acceptor site positioned 3' to the unpaired splice-donor site. Upon homologous recombination between the targeting sequences and genomic DNA, the unpaired-splice-donor site results in splicing to an endogenous splice-acceptor site.

The identification of the targeting event can be facilitated by the use of one or more selectable marker genes typically contained within the targeting DNA construct. In one embodiment, the positive selection marker *neo* is used to select for cells which have a stably incorporated DNA of the targeting construct. The *dhfr* gene is used to subsequently amplify the novel transcription unit present in homologously recombinant cells.

Figure 6 shows a typical arrangement in accordance with the invention of WO '411.

Thus, as with WO '650, WO '411 shows homologous recombination, but there is absolutely no disclosure of target sequences for a site-specific recombinase which flank the heterologous expression control sequence or amplification gene and the positive selection marker gene.

Cruz et al. disclose the homologous recombination of a construct comprising the *neo* gene joined to a *dhfr-ts* gene, to produce cell lines that were DHFR-negative. However, there is absolutely no disclosure in Cruz et al. of containing a sequence for a site-specific recombinase in the construct used therein, as claimed in the present invention.

U.S. '977 is directed to compositions used for targeted modification of genomic DNA using vectors which are homologous with a site subject to endogenous double-stranded staggered restriction. The sequence defining the site is found in the genome at a plurality of sites, which serve as "hot spots" for integration of a sequence homologous to the sequence between two nicks. The sequences are found to have a consensus hexonucleotide sequence TTAAAA, where the nick occurs with greater frequency between T and A, followed by a sequence which has a preference for particular nucleotides at a number of sites. According to U.S. '977, the presence of the nicks in the genomic DNA greatly enhances the efficiency of integration at sites where these nicks occur.

According to column 4, the invention is used to enhance integration of a construct of interest, where there may be little preference for a particular or unique site for integration. Thus, by employing the consensus sequence as part of the construct, one will

greatly enhance the efficiency of integration into a cellular host. By employing known sequences, and by investigating for new sequences, one can build up a library of sequences which will identify "hot spots" for integration throughout the genome of the host.

Although U.S. '977 is not directed to the same field of endeavor as the present case, the reference discloses similar tools for homologous recombination as those used in the present case.

For instance, column 4, line 59 through column 5, line 3, discloses that one may wish to introduce the particular construct at the integration site by using alternative techniques of homologous recombination, such as the in-out technique, where one uses a combination of positive and negative selection markers.

Columns 6-7 of U.S. '977 disclose the various elements that can be used in a transcription cassette. Besides the use of promoters, transcription indication factors, positive selection markers and the like, column 7, lines 31-43, disclose that one may introduce various sequences which allow for specific insertion at the site in the presence of a particular recombinase.

Thus, U.S. '977 discloses many of the tools used in the present invention. However, the reference does not disclose the particular arrangement of these tools, as claimed herein.

Mazure et al. merely teaches the transfection of HIF-1-like sequences. Applicants do not believe that Mazure et al. is particularly relevant to the present invention, outside of this disclosure.

Thus, to summarize the references cited by the Examiner in the rejection, each reference discloses the following:

(1) WO '012 discloses a genetic construct comprising a first compression cassette which comprises a recombinase genetic unit (e.g. a *cre* gene) under the control of a first promoter; and a transgene unit under the control of a second promoter wherein the recombinase genetic unit and the transgene unit are linked, and the first compression cassette is flanked by two recombination loci (e.g. *lox* sites). The first expression cassette is optionally inserted into a second expression cassette such that excision of the first expression cassette from the second expression cassette alters expression of the second expression cassette. Optionally, the genetic construct can further comprise left border and/or right border sequences to facilitate its *in vivo* insertion into chromosomal DNA. The genetic construct is shown, for example, in Figure 5.

While WO '012 shows some aspects of the present invention, the reference does not disclose the presence of a heterologous expression control sequence within the recombination loci, or the important limitation regarding homologous recombination. Instead, WO '012 relies on random integration into the genomic DNA.

(2) WO '650 merely discloses homologous recombination by transfecting cells with a DNA sequence comprising (1) exogenous DNA (e.g., regulatory sequences or DNA repair sequences); (2) sequences homologous with genomic DNA at a preselected site; and (3) amplifiable DNA encoding a selectable marker. There is absolutely no disclosure of the use of target sequences for a site-specific recombinase flanking the exogenous DNA and the selectable marker.

(3) WO '411 discloses a DNA construct comprising (a) targeting sequences; (b) a regulatory sequence; (c) an exon; and (d) an unpaired splice-donor site. There is absolutely no disclosure of target sequences for a site-specific recombinase flanking the regulator sequence and the exon.

(4) Cruz et al. merely teaches the use of homologous recombination to turn a DHFR-positive cell into a DHFR-negative cell. There is absolutely no disclosure of the use of a target sequence for a site-specific recombinase.

(5) U.S. '977 discloses many of the tools used in the present invention, however, U.S. '977 does not teach or suggest the particular arrangement of these elements, as claimed herein. It is also clear from the description and claims of U.S. '977 that this reference is directed to a completely different field of endeavor (i.e., integrating a DNA sequence of interest into a "hot spot" between two nicks in genomic DNA).

(6) Mazure et al. merely teaches the transfection of an HIF-binding nucleic acid, but is otherwise irrelevant to the invention.

Applicants submit that one of skill in the art would not have been motivated to combine the cited references in order to achieve the present invention. The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggest the desirability of the combination. *In re Mills*, 16 USPQ 2nd 1430 (Fed. Cir. 1990).

Applicants first focus on the combination of primary reference WO '012 and secondary reference WO '411, since WO '012 does not disclose homologous recombination and WO '411 does not disclose the use of a site-specific recombinase. One of skill in the art would not combine these references.

The focus of WO '012 is to provide a means to produce genetically-transformed organisms in which the selectable marker genes can be removed, thereby facilitating multiple sequential genetic transformation events using the same selectable marker gene. See the abstract.

Figure 1 shows a construct in accordance with this purpose. The construct in Figure 1A shows plasmid pBS210, which carries a *gusA* gene construct which is rendered inactive by the insertion of the selectable marker gene *nptII* between the promoter Sc4 and the *gusA* gene. The *nptII* marker gene is flanked by two *loxP* sites in direct-repeat configuration. A successful cre/lox -mediated recombination event excises the DNA fragment between the two *loxP* sites, removing the *nptII* cassette and producing the expected recombination test product pBS210a, thereby activating the Sc4 promoter-

derived expression of the *gusA* gene. See pages 43-44 of the specification. This region then becomes available for the insertion of additional gene constructs.

As applicants noted above, WO '012 does not disclose the use of homologous recombination to integrate the DNA construct into the genome of the cell. This is because the purpose of WO '012 is to create a site in the genome containing the target sequences for site-specific recombinase in order to provide for easier integration of additional constructs into the genome. There is absolutely no hint that homologous sequences flanking the expression cassette could be useful, even though the WO '650 reference which discloses the use of homologous recombination was published well before the earliest priority date of WO '012. Thus, applicants submit that one of skill in the art having the WO '012 reference before him, would not be motivated to incorporate the teachings of homologous recombination therein, because there is no hint or suggestion of the desirability of the combination with WO '650.

Regarding WO '411, applicants submit that one of skill in the art, having this reference before, would not modify the reference by adding the teachings of WO '012. This is because WO '411 (as with WO '650) is based on activation of the endogenous gene, by use of exogenous regulatory sequences. There is no teaching in WO '411 or WO '650 of incorporating an exogenous gene into the host genome. On the contrary, as disclosed on page 5, line 32 through page 6, line 7 of WO '411, the methods of the disclosure teach the production of certain proteins by gene activation, in which the coding

DNA sequences of the corresponding protein **are not introduced into the cell by transfection of exogenous DNA encoding the protein**. Thus, the teachings of WO '411 (and WO '650) which are directed to the expression of endogenous DNA would not have been combined with the teaching of WO '012, which is directed to the incorporation of exogenous genes into the host genome.

Regarding U.S. '977, applicants submit that the addition of this reference would not overcome the failure of the WO references to teach the invention, since U.S. '977 does not disclose the unique arrangement of the elements claimed in the present inventions. While U.S. '977 may broadly disclose some of the tools used herein, the reference provides no indication as to how the elements must be arranged. Thus, U.S. '977 cannot help the Examiner to establish a *prima facie* case of obviousness.

The Cruz et al. and Mazure et al. references can only be relied upon for their specific teachings and do not add significantly to the Examiner's rejection. Thus, since none of the previously-noted references would have been combined, as suggested by the Examiner, the addition of Cruz et al. and Mazure et al. would not overcome the failure of the more pertinent teachings to disclose the present invention.

In summary, while the Examiner has cited a number of references containing a hodgepodge of teachings, a sober analysis reveals that these references would have never been combined by one of skill in the art without the benefit of the teachings of the present application. Since such hindsite reasoning is improper, the rejection is improper, and should be withdrawn.

In the event this paper is not timely filed, applicants hereby petition for an appropriate extension of time. The fee for this extension may be charged to our Deposit Account No. 14-1060, along with any other additional fees which may be required with respect to this paper.

Respectfully submitted,
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Enclosure: Sequence Listing

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